bio-protocol Preprint

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Retrograde labeling of motor neurons in adult zebrafish using dextran or DiI

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An abbreviated version of this protocol was published in Science Advances in Apr 2020

Synaptic silencing of fast muscle is compensated by rewired innervation of slow muscle

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Detailed protocol

1. Anesthetize an adult fish in 0.02% tricaine (Tokyo Chemical Industry, Tokyo, Japan).
2. Make a small incision in skin with a surgical blade and expose muscles using tweezers.
3. Prepare a glass needle (Needles for DNA/RNA microinjection can be used).
4. Fill the needle with dextran–Alexa Fluor 488 (molecular weight 10,000; Thermo Fisher Scientific, Waltham, MA) that is dissolved in distilled water (10mg/ml).
5. Connect the needle to Pico Nozzle Kit (World Precision Instruments, Sarasota, FL), mounted on a manipulator (World Precision Instruments).
6. Under stereomicroscope, insert the needle into muscle through an incision in the skin.
7. Gently inject dextran–Alexa Fluor 488 solution by slowly pressing the syringe connected to Pico Nozzle Kit.
8. Transfer the fish to egg water.
9. 5-6 hours after recovery from anesthesia, deeply anesthetize the fish again and decapitate.
10. Dissect the trunk region where dextran solution was injected and fix with 4% PFA/PBS at 4°C overnight.
11. Wash the specimen with PBS and immerse in 30% sucrose/PBS at 4°C overnight.
12. Embed the specimen in O.C.T. compound (Sakura Finetek Japan, Tokto, Japan) and freeze.
13. Perform frontal cryosection of the specimen at 20 µm thickness using a cryostat (CM 3050S; Leica Microsystems, Wetzlar, Germany) and mount onto MAS-GP type A–coated glass slides (Matsunami, Osaka, Japan) and coverslip with DAPI Fluoromount-G (SouthernBiotech, Birmingham, AL) for observation under a Leica TCS SP8 confocal microscope (Leica Microsystems).

For experiments using lipophilic fluorescent tracer DiI [or DiIC18] (Biotium, Fremont, CA)
1. Anesthetize an adult fish in 0.02% tricaine (Tokyo Chemical Industry).
2. Make a small incision in skin with a surgical blade and expose muscles using tweezers.
3. Under stereomicroscope, gently insert crystals of DiI between skin and muscles through an incision in the skin, with a glass needle or tweezers.
4. Transfer the fish to egg water.
5. 24 hours after recovery from anesthesia, deeply anesthetize fish for decapitation.
6. Follow steps 10-13 described above.

Solution

EggWater

Sea salt	120mg
Distilled water	2L
1%methylene blue	200µL

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Zempo, B. and Ono, F. (2020). Retrograde labeling of motor neurons in adult zebrafish using dextran or DiI. Bio-protocol Preprint. bio-protocol.org/prep547.
2. Zempo, B., Yamamoto, Y., Williams, T. and Ono, F.(2020). Synaptic silencing of fast muscle is compensated by rewired innervation of slow muscle . Science Advances 6(15). DOI: 10.1126/sciadv.aax8382

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